RESULTS OF PHYSIOLOGICAL AND ECOLOGICAL INVESTIGATIONS OF CHLORELLA CULTURES AS A LINK IN A CLOSED ECOLOGICAL SYSTEM*

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The use of the principle of biological circulation of matter as a means of life /451
support for the crew of spacecraft was suggested by K. E. Tsiolkovskiy [13] as a natural continuation of his work on the theoretical basis of interplanetary flights. It is worth noting that the first attempts at an experimental approach to this problem were undertaken as long ago as in 1915-1919 by A. F. Tsander, an engineer who was one of the founders of rocket construction in the USSR. In experiments to grow vegetables on artificial soil substitutes (charcoal), he used human metabolic products for their nutrition [12]. These were evidently the first experimental ecological investigations directly aimed at meeting astronautical needs.

At the present time the idea of creating a closed cycle of circulation of matter is not only a subject of experimental research, but is also embodied in design work undertaken by many laboratories in several different countries.

Among the first components suggested for biological life-support systems in spacecraft were unicellular algae and, in particular, the genus Chlorella, which is now regarded as a probable element in the photosynthetic link included in the /452
closed ecological system.

This paper describes a study mainly of the material balance of that part of the photosynthetic link which can be based on the use of unicellular algae. When this problem is examined certain general aspects of the simulation of ecological systems must not be overlooked, for they determine the main pathways to be followed in the development of individual functional links. In contrast to living nature, which at all stages of its evolution was an integrated system and never appeared by synthesis from preexisting elements, we are apparently compelled to follow a path of "one-act creation," creating a functionally intact system from existing elements adapted for the performance of function when incorporated in another, already acting system, nature as it exists on Earth.

Because of the unprecedented complexity of this problem, the choice of a general method of solution is extremely important. Obviously the method of direct synthesis by trial and error is unsuitable here because of the extreme complexity and the not always predictable volume of direct connections and feedbacks between the elements of the constituted system. In this case the only suitable method is by the planned construction of a system as a whole and of its corresponding

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components on the basis of preliminary calculations. In other words, the closed ecological system must be created from the very beginning as an integrated system with perfectly definite tasks and functional characteristics, determining its composition and structure and the main parameters of its component elements.

It follows from what has been said that the design of experimental models of closed ecological systems is largely based on the study and quantitative description of functional connections between preliminarily selected objects which determine their position, their requirements and their external work in the system of rotation of matter and energy, their ecological "niche."

We must therefore study and describe the input and output characteristic of the hypothetical elements of the future system. Such characteristics are an essential condition for the preliminary calculation and the practical accomplishment of models of biological systems for any definite purpose.

The following principal characteristics may be stipulated, as essential for calculations and model construction, for the link consisting of unicellular algae, the subject of this report.

A. Input parameters.

Energy: Optimal and extreme values of intensity and of spectral composition; intake regime, specific consumption.

General conditions of function: Optimal and extreme values of temperature, acidity of medium, dynamic conditions of cultivation—mechanical overloads, pressure, and their differentials.

Material requirements: Optimal and extreme values of concentration of carbon dioxide and mineral food elements, their specific consumption.

B. Output parameters.

Energy: The free energy available in the system expressed as a ratio of the incident and absorbed energies.

Material output: Specific productivity with respect to oxygen and biomass, chemical composition and food value of biomass, its coefficient of useful utilization in the system.

Side processes: Pollution of the atmosphere with incidential contaminants, accumulation of products of cell metabolism and destruction in the nutrient medium.

In addition, the provision of automatic control in the system requires the study of some special processes including the general properties of the link in the system of information and control: the informative value of individual parameters of the link, frequency characteristics of spontaneous and forced oscillations, the parameters of transitional processes and transmission functions, etc.

This report summarizes briefly the results of an investigation of characteristics of the material balance of Chlorella cultures essential for the determination of their place and their requirements and of the role of matter.

Data are also given relative to the dynamic properties of Chlorella cultures as an object for control.

In the Soviet Union the study of cultures of unicellular algae for practical use in air regeneration systems was founded on the investigations of N.S. Gayevskaya, V.V. Pinevich, V.A. Chesnokov, N.N. Verzilin and others [1, 10, 14], and also on work by V.Ye. Semenenko, M.G. Vladimirova and A.A. Nichiporovich [11].

The first attempts at supporting the gas exchange of animals and man by photosynthesis of Chlorella in closed gas-exchange systems were made in 1960-1961 (G.I. Meleshko, Ye.K. Lebedeva, T.B. Galkina and others). Later they carried out complex investigations of Chlorella cultures as a possible link in a closed ecological system.

The first experiments on the support of animal gas exchange led to the conclusion that the successful experimental simulation of ecological systems requires the most accurate knowledge possible of the material and energy requirements, the conditions of function, and the material outputs of each element of the system.

The direct application of physiological data to the creation of a stable and highly productive biotechnologic process in populations of algae is hampered by the fact that their general physiological characteristics do not allow for the transforming effect of the population on the habitat. At the same time, as a result of this effect, the external environmental conditions do not always remain constant within the population but deviate from the initial value at the borders of the territory which it occupies (the biotope). The greater the density of the population and its specific productivity, the greater this deviation. /454

Because of this factor, intrapopulation gradients of the properties of the medium arise, and this is an important feature distinguishing the population as a system from a simple multiplicity of individuals. For the quantitative assessment of functional connections of a given link in the ecological system it therefore became necessary to organize special experimental ecological investigations using as object a highly productive population of the species under study, with all the special features of the intrapopulation relationships of individual organisms and the relationship of the population as a whole with the surrounding medium.

To obtain the essential data, a group of workers under G.I. Meleshko's direction carried out combined investigations of the material balance of Chlorella when cultivated in high-productivity photosynthetic reactors with a closed air system [3, 6-9].

An important characteristic of the photosynthetic link is the gas-exchange coefficient: the ratio between the volume of carbon dioxide utilized and the volume of oxygen produced, which must correspond to the respiratory quotient of the consumer links of the ecological system. Experimental determination of

this value was based on direct measurements of the carbon dioxide and oxygen balance in a hermetically sealed system. The value obtained was 0.79 ± 0.01 with considerable dispersion (standard deviation 0.066).

The gas-exchange coefficient was found to be independent of density of the suspension within the range from 3×10^8 to 1×10^{10} cells/ml and of the intensity of photosynthesis within the range from 0.15 to 6.0 liters CO_2/h from one liter of suspension. This level of the coefficient of gas exchange was obtained during cultivation of Chlorella on Tamiya's medium in which nitrates were the source of nitrogen.

In cases when the source of nitrogen in the medium was urea, the coefficient of gas exchange rose to 0.995 ± 0.022. When algae were cultivated on liquid and solid products of human metabolic activity, preliminarily mineralized in an aerobic bacterial reactor, the coefficient of gas exchange moved closer to its level on nitrate media, namely to 0.82 [8]. As the results show, the coefficient of gas exchange of a Chlorella culture on mineral and mineralized organic media /455 lies within the usual limits of the human respiratory quotient.

TABLE 1. Relationship between Increase in Biomass, Absorption of CO_2 and Liberation of Oxygen in Intensive Chlorella Culture

Density of suspension, g/liter	Increase in biomass, g dry weight	Absorption of CO_2, liters/g dry weight	Liberation of O_2, liters/g dry weight
2,2	0,19	1,00	1,23
3,75	0,28	0,97	1,11
4,5	0,41	1,03	1,20
5,6	0,29	0,97	1,21
6,75	0,42	1,04	1,24
11,2	0,32	1,00	1,11
18,0	0,46	1,04	1,38
25,7	0,47	1,02	1,36
33,0	0,60	0,98	1,23
$M \pm m$	—	1,00±0,01	1,23±0,03

Commas represent decimal points

Later, in a special series of investigations, a simultaneous study was made of the intensity of photosynthesis, the gas balance and mineral feeding of algae, and their elementary and biochemical composition. Some of these results are shown in Table 1, in which it can be seen that during the formation of 1 gram dry

substance of algae on the average 1 liter carbon dioxide is absorbed and 1.23 liters oxygen liberated (at 20°). This ratio is maintained over a fairly wide range of densities of the culture, intensities of photosynthesis, pH of the medium and intensities of illumination.

The high degree of correlation between the studied indices of intensity of photosynthesis means that any one of them is suitable as a criterion of productivity of the culture and as the source of informative signals for the automatic control of the principal growth conditions of the algal culture. Accordingly, in subsequent experiments an automatic carbon dioxide gas analyzer with positioned regulation was successfully used.

At the same time and in the same experiment the dynamics of extraction of mineral feeding elements from the culture medium and the accumulation of these elements in the algal cells (Table 2) and the biochemical composition of the cell matter were investigated [3, 6].

Comparison of loss of elements from the nutrient medium and their accumulation in the biomass shows that a proportion of them is resecreted into the culture medium, probably in the form of products of cell metabolism or destruction, /456 constituting a relatively small amount in the case of a highly productive culture.

TABLE 2. Accumulation of Principal Elements in Algal Cells

Element	In mg/g dry substance	As ratio of loss from medium, %	Element	In mg/g dry substance	As ratio of loss from medium, %
Nitrogen. . . .	80,0	96,5	Magnesium . .	5,7	99,1
Phosphorus. .	17,4	98,9	Iron.	0,3	—
Sulphur	11,2	—	Carbon.	500,0	92,4
Potassium . .	16,0	—			

Commas represent decimal points.

The Chlorella biomass obtained under these conditions had an empirical formula $C_{6.6}H_{12.2}O_{3.5}N_{1.0}$ and contained: ash 7.3% of dry weight, protein 56.3%, fat 8.5%, carbohydrates including cellulose 35.2% of the organic matter.

Combustion of a substance with the formula indicated above theoretically gives a gas balance in close agreement with that obtained experimentally.

As a result of their investigations these authors obtained complex characteristics for an intensive Chlorella culture reflecting the quantitative relationship between the gas balance of the culture, the consumption of mineral feeding elements from the culture medium, their accumulation in the cells, and production of a biomass of definite composition. The results given above were obtained during

intensive cultivation conditions. This was made possible by preliminary investigation of the optimal conditions of cultivation of algae in high-density populations.

One object of the investigations was to discover the combination of conditions for cultivation of algae capable of maintaining a high level of specific productivity of their suspension. Such a combination was found and put into effect in cultivation by the film method, by means of which under laboratory conditions the productivity of the culture could be increased to 8-10 liters oxygen from one liter of suspension per hour, i.e., values commensurate with the potential capabilities of the Chlorella culture were obtained [7].

The achievement of such a high level of productivity was also made possible by the fact that optimal levels of the principal condition of cultivation were revised to meet the specific properties of high-density populations. In particular, experiments by G.I. Meleshko and L.M. Krasotchenko revealed higher levels of saturating concentrations of carbon dioxide than those obtained in modern laboratory practice, reaching (in air) 1.5-1.8 and 4.5-5.5% for suspension densities of $0.5\text{-}1 \times 10^9$ and $4\text{-}5 \times 10^9$ cells/ml respectively [8]. /457

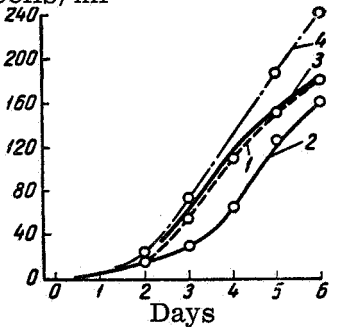

Figure 1. Growth of Culture of Algae on Freshly Prepared and Reused Medium:

1) Control (Freshly Prepared Medium); 2) Experiment (Reused Medium Without Dilution; 3 and 4) After Dilution 1:1 and 1:3.

When algae are cultivated in suspensions of high specific productivity, the accumulation of biologically active products in the culture medium becomes of practical importance. In some interesting experiments T.B. Galkina [2] showed that with an increase in density of the suspension, the initially stimulant action of the culture medium diminishes and then changes into an inhibitory action. The transition point in this case is the "physiological age" of this medium at which about 500 million cells will grow per milliliter irrespective of the actual cultivation time.

When the experiment was performed in reverse, serial dilutions were made of the same medium as that which exhibited an inhibitory action. This showed that the magnitude of the inhibitory effect diminshes with dilution, and with further dilution changes into a stimulant effect. These results gave conclusive evidence that the stimulants and inhibitory action of substances accumulating in the medium during prolonged cultivation of algae are of the same nature (Fig. 1).

In a series of experiments in this group of investigations the content of certain gaseous contaminants was determined in the closed air circuit of the reactor [5]. Besides concerning existing data for the liberation of carbon monoxide by the Chlorella culture [15, 17, 19, 20], these experiments also showed that the specific accumulation of this gas is inversely proportional to the intensity of photosynthesis in the culture (Table 3).

The results suggest that carbon monoxide formation is not directly connected with the photosynthetic process. By analogy with the formation of endogenous

carbon monoxide in the human body [20], its formation during cultivation of algae may be associated with the oxidative destruction of the tetrapyrrole ring of the chlorophyll molecule.

TABLE 3. Accumulation of Carbon Monoxide per Gram Increase in Dry Matter of Chlorella in Cultures of Different Productivities (Tamiya's medium)

Increase in dry substances per liter suspension, g/liter, h	Accumulation of CO, mg/g dry substance (M ± m)	Number of experiments	Increase in dry substances per liter suspension, g/liter, h	Accumulation of CO, mg/g dry substance (M ± m)	Number of experiments
0,04 (0,003–0,085)	2,07±0,05	7	0,30 (0,170–0,606)	0,092±0,24	7 11

Commas represent decimal points.

Parallel to this series of investigations, Ye. A. Ivanov and I. V. Aleksandrova /458 studied the dynamics and frequency characteristics of an algal suspension as an object for control [4]. By means of a low-inertia polarographic method these workers showed that a stepwise change in the intensity of illumination induces a negative (opposite) phase in the photosynthetic reaction in 97% of cases. The mean duration of this phase is 3 min, after which the intensity of photosynthesis settles down to correspond to the new level of illumination. The total duration of the transitional process is 8-12 min. A subsequent study of the relationship between the parameters of the transitional process and conditions of cultivation enabled these authors to deduce an analytical expression for transitional functions of a Chlorella culture depending on illumination.

These workers also showed that the use of the negative phase of the photosynthetic reaction during a stepwise change in illumination for the automatic search for the extreme value of photosynthesis and for bringing the culture away from this value can shorten the time required for this purpose by 5-6 times (to 50-60 sec) compared with a search based on the direct reaction.

The results obtained by these workers reflect the demands made by the Chlorella suspension on the high-speed parameters of the automatic control system based on illumination and they indicate ways of increasing the rapidity of action of automatic systems. Solution to problems of automatic control of algal cultures is inconceivable without investigation of the characteristics of self-oscillating processes in a controllable system. This problem was first considered and analyzed by Ivanov and Aleksandrova on the basis of an extensive experimental material [4] (see also the present collection). They found that spontaneous changes in photosynthesis arise fairly rarely and the probability of their appearance was 0.18. The mean value of the deviation of intensity of photosynthesis from its mean under concrete experimental conditions was ± 21% and the mean rate of deviation was 6.75%/hour. In some experiments a transition to a steady decrease /459 in intensity of photosynthesis was observed with a mean rate of 3-4%/day.

We consider that the results of such investigations, besides being of immediate importance, are also of considerable interest as general criteria for the comparative assessment of the technologic quality of methods and apparatus used for cultivation of algae.

The complex character of these investigations in high-productivity reactors made it possible to obtain extensive physiological, ecological and technological characteristics of the algal culture. These characteristics are essential both for organization of high-productivity technology and also for the algal link when simulated in isolated gas-exchange systems and closed systems of rotation of matter.

Probably these results constitute that minimal volume of information essential with respect to any object included in an ecological system. Only if such data are available for each hypothetical link in the system can we consider the possibility of planned design of experimental ecological models and also the possibility of subsequent study of complex relationship between closed systems as a fundamental task in their experimental ecology.

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